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(54) **MIXED GAS MICROBUBBLE COMPOSITIONS**

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(51) **Int. Cl.**[7] **A61B 8/00**

(52) **U.S. Cl.** **424/9.52**

(58) **Field of Search** 424/9.51, 9.52; 128/662.02; 600/458, 441

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(57) **ABSTRACT**

A microbubble preparation formed of a plurality of microbubbles comprising a first gas and a second gas surrounded by a membrane such as a surfactant, wherein the first gas and the second gas are present in a molar ratio of from about 1:100 to about 1000:1, and wherein the first gas has a vapor pressure of at least about (760–x) mm Hg at 37° C., where x is the vapor pressure of the second gas at 37° C., and wherein the vapor pressure of each of the first and second gases is greater than about 75 mm Hg at 37° C.; also disclosed are methods for preparing microbubble compositions, including compositions that rapidly shrink from a first average diameter to a second average diameter less than about 75% of the first average diameter and are stabilized at the second average diameter; kits for preparing microbubbles; and methods for using such microbubbles as ultrasound contrast agents.

26 Claims, No Drawings

stearate was warmed to approximately 45° C., to speed the dissolution of the lactose, before injecting 1.5 ml of the warmed solution into the vial. The vial was then gently agitated by inversion for approximately 30 seconds to dissolve the lactose before injecting the microbubbles thus prepared into the Horiba LA-700 particle analyzer. A 7.7 micron volume weighted median diameter was measured approximately one minute after dissolution. The diameter of these microbubbles remained nearly constant, changing to a median diameter of 7.1 microns in 10 minutes. When the experiment was repeated with air filled lactose, the particle analyzer gave only background readings one minute after dissolution, thus demonstrating that gas osmotically stabilized microbubbles can be produced by the dissolution of gas-filled cavity-containing structures.

EXAMPLE VI

Preparation of Larger Bubbles that Shrink to a Desired Size

Microbubbles with an average volume weighted size of 20 microns shrinking to 2 microns were prepared by sonication of an isotonic aqueous phase containing 2% Pluronic F-68 as the surfactant, CO_2 as a diluent gas and perfluorohexane as the gas osmotic agent.

In this experiment, 1.3 ml of a sterile water solution containing 0.9% NaCl, 2% Pluronic F-68 and 1% sucrose stearate was added to a 2.0 ml vial. The vial had a remaining head space of 0.7 ml initially containing air. A mixture of air saturated with perfluorohexane at 25 degrees C. diluted by a factor of 10 with CO_2 (684 Torr CO_2+54 Torr air+22 Torr perfluorohexane) was used to flush the head space. The vial was sealed with a thin 0.22 mm PTFE septum. The vial was sonicated as in Example I, forming a white solution of finely divided microbubbles, having an average particle size of 28 microns as measured by Horiba LA-700 laser light scattering analyzer. In the 4% dextrose +0.25mM NaOH solution of the Horiba, the average bubble diameter rapidly shrank in 2 to 4 minutes from 28 microns to 5 to 7 microns, and then remained relatively constant, reaching 2.6 micron after 27 minutes. This is because the CO_2 leaves the microbubbles by dissolving into the water phase.

EXAMPLE VII

Perfluoroheptane Stabilized Microbubble in vitro Experiment

Microbubbles were prepared as in Example I above employing perfluoroheptane saturated air (75 torr plus 685 torr air) and were measured as in Example II above. The average number weighted diameter of these microbubbles was 7.6 micron, one minute after circulation, and 2.2 microns after 8 minutes of circulation. This persistence, compared to the near immediate disappearance of microbubbles containing only air, demonstrates the gas osmotic stabilization of perfluoroheptane.

EXAMPLE VIII

Perfluoropropionyl Amine Stabilized Microbubble in vivo Experiment

Microbubbles were prepared as in Example I above, employing perfluorotripropyl amine saturated air and were assessed as in Example III above. The usable vascular persistence of these microbubbles was found to be 2.5 minutes, thus demonstrating the gas osmotic stabilization of perfluorotripropyl amine.

EXAMPLE IX

Effect of a Non Newtonian Viscoelastic Surfactant–Sucrose Stearate

Microbubbles were prepared as in Example I above employing 0.9% NaCl, 2% Pluronic F-68 and 2% sucrose stearate as the surfactant and with perfluoropropane saturated air and perfluorohexane saturated air in the headspace. These two preparations were repeated with the same surfactant solution minus sucrose stearate. All four microbubble preparations were assessed as in Example III above. The usable vascular persistence of these microbubbles are listed below:

2% Pluronic P-68+2% sucrose stearate persistence
- 2 minutes perfluoropropane
- 4 minutes perfluorohexane

2% Pluronic F-68 only persistence
- 2 minutes perfluoropropane
- 1 minute perfluorohexane

As seen above, the reduced surface tension made possible by the non-Newtonian viscoelastic properties of sucrose stearate prevented the less volatile perfluorohexane from condensing, allowing perfluorohexane microbubbles of longer persistence to be produced.

The foregoing description details certain preferred embodiments of the present invention and describes the best mode contemplated. It will be appreciated, however, that no matter how detailed the foregoing appears in text, the invention can be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

What is claimed is:

1. An injectable ultrasound contrast medium comprising:
(a) biocompatible at body temperature gaseous substances which, when in suspension in an aqueous carrier liquid containing surfactants, additives, and stabilizers, provide contrast agents for ultrasound echocardiography, wherein:
(b) the medium is a mixture which contains a modifier gas A and the balance of the mixture is an osmotic agent gas B;
(c) the ratio of gas A to gas B is 200:1 to 3:2 by volume; and
(d) gas B has a water solubility of not more than about 0.5 mM at 25° C. and one atmosphere and an average molecular weight at least about 4 times that of gas A.

2. An injectable ultrasound contrast medium comprising:
(a) biocompatible at body temperature gaseous substances which, when in suspension in an aqueous carrier liquid containing surfactants, additives, and stabilizers, provide contrast agents for ultrasound echocardiography, wherein:
(b) the medium is a mixture which contains a modifier gas A and the balance of the mixture is an osmotic agent gas B;
(c) the ratio of gas A to gas B is 738:22 to 3:2 by volume; and
(d) gas B is selected from the group consisting of fluorocarbons, CCl_2F_2, $CHClF_2$, $N(C_2F_5)_3$, $N(C_3F_7)_3$, and mixtures thereof.

3. The ultrasound contrast medium of claim 1 wherein gas B is a fluorocarbon biocompatible gas.

4. The ultrasound contrast medium of claim 3 wherein the fluorocarbon biocompatible gas is selected from the group consisting of CF_4, C_3F_8, C_4F_8, C_4F_{10}, C_5F_{10}, C_5F_{12}, and mixtures thereof.

5. The ultrasound contrast medium of claim 4 wherein the fluorocarbon gas is octafluorocyclobutane.

6. The ultrasound contrast medium of claim 2 wherein gas A is selected from the group consisting of air, oxygen, nitrogen, carbon dioxide, and mixtures thereof.

7. An injectable ultrasound contrast agent comprising:

(a) a suspension of gas filled microbubbles or microballoons in a physiologically acceptable aqueous carrier comprising surfactants, additives, and stabilizers, wherein:

(b) the gas is a gas mixture containing a biocompatible gas A and the balance of the mixture is a biocompatible gas B;

(c) the ratio of gas A to gas B is 200:1 to 3:2 by volume; and

(d) gas B has a water solubility of not more than about 0.5 mM at 25° C. and one atmosphere and an average molecular weight at least about 4 times that of gas A.

8. An injectable ultrasound contrast agent comprising:

(a) a suspension of gas filled microbubbles or microballoons in a physiologically acceptable aqueous carrier comprising surfactants, additives, and stabilizers, wherein:

(b) the gas is a gas mixture containing a biocompatible gas A and the balance of the mixture is a biocompatible gas B;

(c) the ratio of gas A to gas B is 738:22 to 3:2 by volume; and

(d) gas B is selected from the group consisting of fluorocarbons, CCl_2F_2, $CHClF_2$, $N(C_2F_5)_3$, $N(C_3F_7)_3$, and mixtures thereof.

9. The ultrasound contrast agent of claim 7 wherein gas B is a fluorocarbon biocompatible gas.

10. The ultrasound contrast agent of claim 9 wherein the fluorocarbon biocompatible gas is selected from the group consisting of CF_4, C_3F_8, C_4F_8, C_4F_{10}, C_5F_{10}, C_5F_{12}, and mixtures thereof.

11. The ultrasound contrast agent of claim 9 wherein gas A is selected from the group consisting of air, oxygen, nitrogen, carbon dioxide, and mixtures thereof.

12. The ultrasound contrast agent of claim 10 wherein the surfactants comprise at least one film forming surfactant and, optionally, stabilizers.

13. The ultrasound contrast agent of claim 12 wherein the film forming surfactant is a phospholipid.

14. The ultrasound contrast agent of claim 7 wherein the surfactant is polyethylene oxide sorbitan fatty acid ester or sorbitol.

15. A dry formulation comprising:

(a) surfactants, additives, and stabilizers stored under a mixture of substances which at body temperature are biocompatible gases, wherein:

(b) at least one of the biocompatible gases is a fluorine-containing gas having a water solubility of not more than about 0.5 mM at 25° C. and one atmosphere and an average molecular weight at least about 4 times that of air, oxygen, nitrogen, carbon dioxide or mixtures thereof;

(c) the balance is air, oxygen, nitrogen, carbon dioxide, or mixtures thereof; and

(d) the ratio of said air, oxygen, nitrogen, carbon dioxide, or mixtures thereof to said fluorine-containing gas is 200:1 to 3:2 by volume.

16. A dry formulation comprising:

(a) surfactants, additives, and stabilizers stored under a mixture of substances which at body temperature are biocompatible gases, wherein:

(b) at least one of the biocompatible gases is a fluorocarbon gas;

(c) the balance is air, oxygen, nitrogen, carbon dioxide, or mixtures thereof; and

(d) the ratio of said air, oxygen, nitrogen, carbon dioxide, or mixtures thereof to said fluorine-containing gas is 738:22 to 3:2 by volume.

17. A two component kit comprising:

(a) as the first component, a dry formulation of surfactants, additives, and stabilizers stored under a mixture of substances which at body temperature are gases; and,

(b) as the second component, a physiologically acceptable carrier liquid which, when admixed with the first component, provides, as a suspension of the two components, an ultrasound contrast agent, wherein:

(c) at least one of the gases in the mixture is a gas having a water solubility of not more than about 0.5 mM at 25° C. and one atmosphere and an average molecular weight of at least about 4 times that of air, oxygen, nitrogen, carbon dioxide, or mixtures thereof;

(d) the balance is air, oxygen, nitrogen, carbon dioxide, or mixtures thereof; and

(e) the ratio of said air, oxygen, nitrogen, carbon dioxide, or mixtures thereof to said gas having a water solubility of not more than about 0.5 mM at 25° C. and one atmosphere and an average molecular weight of at least about 4 times that of air, oxygen, nitrogen, carbon dioxide, or mixtures thereof is 200:1 to 3:2 by volume.

18. A two component kit comprising:

(a) as the first component, a dry formulation of surfactants, additives, and stabilizers stored under a mixture of substances which at body temperature are gases; and,

(b) as the second component, a physiologically acceptable carrier liquid which, when admixed with the first component, provides, as a suspension of the two components, an ultrasound contrast agent wherein:

(c) at least one of the gases in the mixture is a fluorocarbon biocompatible gas;

(d) the balance is air, oxygen, nitrogen, carbon dioxide, or mixtures thereof; and

(e) the ratio of said air, oxygen, nitrogen, carbon dioxide, or mixtures thereof to said gas having a water solubility of not more than about 0.5 mM at 25 ° C. and one atmosphere and an average molecular weight of at least about 4 times that of air, oxygen, nitrogen, carbon dioxide, or mixtures thereof is 200:1 to 3:2 by volume.

19. The two component kit of claim 18 wherein the fluorocarbon biocompatible gas is selected from the group consisting of CF_4, C_3F_8, C_4F_8, C_4F_{10}, C_5F_{10}, C_5F_{12}, and mixtures thereof.

20. A method of imaging organs in a living body, said method comprising the steps of:

(1) administering to said body an ultrasound contrast agent comprising a suspension of gas filled microbubbles or microballoons in a physiologically acceptable aqueous carrier comprising surfactants, additives, and stabilizers, wherein:

(a) the gas is a gas mixture containing a biocompatible gas A and a biocompatible gas B;

(b) gas B has a water solubility of not more than about 0.5 mM at 25° and one atmosphere and an average molecular weight at least about 4 times that of gas A, and

(c) the ratio of gas A to gas B is 200:1 to 3:2 by volume; and

(2) subjecting said body to ultrasound therapy.

21. A method of imaging organs in a living body, said method comprising the steps of:

(1) administering to said body an ultrasound contrast agent comprising a suspension of gas filled microbubbles or microballoons in a physiologically acceptable aqueous carrier comprising surfactants, additives, and stabilizers, wherein:

(a) the gas is a gas mixture containing a biocompatible gas A and a biocompatible gas B;

(b) gas B is a fluorocarbon; and

(c) the ratio of gas A to gas B is 738:22 to 3:2 by volume; and

(2) subjecting said body to ultrasound therapy.

22. The method of claim **21** wherein the flourocarbon biocompatible gas is selected from the group consisting of CF_4, C_3F_8, C_4F_8, C_4F_{10}, C_5F_{10}, C_5F_{12}, and mixtures thereof.

23. The method of claim **21** wherein gas A is selected from the group consisting of air, oxygen, nitrogen, carbon dioxide, and mixtures thereof.

24. The method of claim **21** wherein the surfactants comprise at least one film forming surfactant and, optionally, stabilizers.

25. The method of claim **24** wherein the film forming surfactant is a phospholipid.

26. The method of claim **20** wherein the surfactant is a polyethylene oxide sorbitan fatty acid ester or sorbitol.

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